Running Title: Effect of CO_2 sequestration on foraminifera

Impact of intentionally injected carbon dioxide hydrate on deep-sea benthic foraminiferal survival

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Abstract

Sequestration of carbon dioxide (CO_2) in the ocean is being considered as a feasible mechanism to mitigate the alarming rate in its atmospheric rise. Little is known, however, about how the resulting hypercapnia and ocean acidification may affect marine fauna. In an effort to understand better the protistan reaction to such an environmental perturbation, the survivorship of benthic foraminifera, which is a prevalent group of protists, was studied in response to deep-sea CO_2 release. The survival response of calcareous, agglutinated, and thecate foraminifera was determined in two experiments at ~3.1 and 3.3 km water depth in Monterey Bay (California, USA). Approximately five weeks after initial seafloor CO_2 release, in situ incubations of the live-dead indicator CellTracker Green were executed within seafloor-emplaced pushcores. Experimental treatments included direct exposure to CO_2 hydrate, two levels of lesser exposure adjacent to CO_2 hydrate, and controls, which were far removed from the CO_2 hydrate release. Results indicate that survivorship rates of agglutinated and thecate foraminifera were not significantly impacted by direct exposure but the survivorship of calcareous foraminifera was significantly lower in direct exposure treatments compared to controls. Observations suggest that, if large scale CO_2 sequestration is enacted on the deep-sea floor, survival of two major groups of this prevalent protistan taxon will likely not be severely impacted, while calcareous foraminifera will face considerable challenges to maintain their benthic populations in areas directly exposed to CO_2 hydrate.

Introduction

In both the scientific and public arenas, much attention has recently focused on the issues of global warming and climate change. Although debate over the most appropriate mitigation pathway continues, it is certain that the atmospheric concentration of carbon dioxide (CO_2) has increased significantly in the recent past (e.g., Keeling *et al*., 1995; Keeling 1998). One option to curtail the rapidly rising atmospheric CO_2 levels under consideration is to sequester waste CO_2 in the deep ocean (e.g., Caldeira, Akai *et al*., 2005). A number of scenarios have been proposed for such ocean carbon storage, including fertilization of the sea surface with iron to promote phytoplankton growth and accelerate the biological pump, thereby increasing dissolved inorganic carbon (DIC) export to the deep sea (e.g., Buesseler *et al*., 2004), CO_2 injection at mid-ocean water depths (e.g., Ozaki, 1997), and CO_2 injection onto the deep-sea floor (e.g., Brewer *et al*., 2000). Ongoing research is attempting to elucidate the benefits and drawbacks of each approach if implemented on the large scale. In particular, impacts on both ocean chemistry and inhabitants must be ascertained, especially given that CO_2 dissolution causes a concomitant decrease in pH (Brewer *et al*., 2000). Physiological responses to elevated CO_2, or hypercapnia, and ocean acidification are challenging to organisms in general (reviewed in, e.g., Siebel & Walsh, 2003; Pörtner *et al*., 2004); it is unclear which taxa, if any, will be unaffected by these environmental pressures.

A series of in situ experiments have been conducted to assess the effects of direct injection of CO_2 on the seafloor, concentrating on the lower bathyal zone since it theoretically provides a longer period of sequestration compared to shallow water (Brewer *et al*., 1999, 2000; Barry *et al*., 2005). Although studies have begun to describe the effects of such CO_2 disposal on deep-sea fauna including fish (Tamburri *et al*., 2000), crustaceans (Barry *et al*., *submitted*),

echinoderms (Barry *et al*., *submitted*), and metazoan meiofauna (e.g., Carman *et al*., 2004; Watanabe *et al*., 2006; Fleeger *et al*., 2006; Thistle *et al*., 2007), little is known about the effect of such activities on protista. Because protists comprise a substantial portion of the deep-sea benthos (e.g., Alongi & Pinchon, 1988; Coull *et al*., 1977; Snider *et al*., 1984; Gooday *et al*., 2000; Smith *et al*., 2002), it is important to establish the effects of bathyal CO_2 release on these single-celled eukaryotes. One study that surveyed the effects of CO_2 release on deep-sea meiobenthos observed that 4.5 weeks after seafloor CO_2 injection many meiofaunal groups (i.e., nematode metazoans and flagellate and amoebae protists) experienced elevated mortality compared to sites removed from CO_2 manipulation (Barry *et al*., 2004). Robust conclusions about the response of some meiofaunal protists (i.e., allogromiid [thecate] foraminifera and ciliates) to that increased pCO_2 exposure could not be drawn, however, due to low population sizes (Barry *et al*., 2004) and because an accurate means to determine survival was not implemented for those taxa.

In a continuing effort to better ascertain the response of meiofaunal protists to elevated CO_2 exposure, a study was executed to determine in situ survival response of deep-sea benthic foraminifera to CO_2 release simulating seafloor disposal of this greenhouse gas. For the study, two experiments were conducted, each with a duration of 4.5 weeks in order to evaluate faunal responses to quasi-chronic changes in ocean chemistry, rather than ephemeral changes over short periods (i.e., days). Foraminifera were selected as the study taxon for two main reasons. First, they are a critical link in marine food webs (e.g., Legendre & Le Févre, 1995; van Oevelen *et al*., 2006; Rowe *et al*., *in press*). Second, while a large proportion of foraminiferal species secrete calcium carbonate shells called tests, the majority lack inorganic tests (so-called allogromiid or thecate forms, e.g., Gooday, 2002) or use detrital particles to construct agglutinated tests (Sen

89 Gupta, 1999), allowing comparative experimentation between calcifying and non-calcifying
90 species of the same taxonomic group of Rhizarian protists (Adl *et al*., 2005). This diversity in
91 foraminiferal test composition is particularly useful when considering the biologic effects of
92 ocean acidification. It may be hypothesized that the mortality of calcareous foraminiferal species
93 will be higher in response to deep-sea CO_2 sequestration than the mortality of species of the two
94 non-carbonate foraminiferan groups (i.e., thecate and agglutinated taxa).

Materials and Methods

Two replicate experiments were conducted on the seafloor of Monterey Bay, off the California coast (USA). Using the surface support vessel RV *Western Flyer* and the ROV *Tiburon* for each experiment, a set of 40.6-cm diameter PVC cylinders were placed on the seafloor so that ~15 cm extended above the sediment-water interface; 15 cylinders (Experiment 1) and 7 cylinders (Experiment 2) were configured in a circle with a diameter of ~ 20 m. Then, over two to three days, liquid CO_2 was injected into each cylinder using methods described in, e.g., Barry *et al*. (2004), Carman *et al*. (2004), and Fleeger *et al*. (2006). CO_2 hydrate formed immediately as a 'skin' on liquid CO_2 pools. The targeted pH decline within the ~20-m diameter circle compared to in situ pH (~7.8; Thistle *et al*., 2007) was 0.2 pH units because data suggests that pH declines of that magnitude can be an important physiological threshold (e.g., Seibel & Walsh, 2003).

For both experiments, Conductivity-Temperature-Depth instruments (SeaBird Model 19+ CTDs) were deployed on the seabed at locations near the margin, ~3-5 m from the margin, and in the center of the circle of CO_2 cylinders (Fig. 1). Each CTD was equipped with up to 4 Seabird Model SBE 18 pH sensors, positioned from 3 to 50 cm above the seabed. These instruments collected data at ~2 minute intervals throughout each experiment. Perturbations in the pH of surficial sediments were also measured during Experiment 2 within CO_2 cylinders and from 0.1 - 8 m from CO_2 pools, as reported in Barry *et al*. (*submitted*).

During the commencement of Experiment 1, a suite of control samples was obtained, as explained below. The first experiment was initiated from 13-17 December 2004 at a water depth of 3088 m (36.6985^{o}N, 123.0020^{o}W) and terminated from 18-21 January 2005. Experiment 2 was initiated from 12-16 December 2005 at a water depth of 3266 m (35^{o}48.6105'N,

118 122°34.0952' W) and terminated from 18-21 January 2006. The carbonate chemistry in the
119 region of the experimental sites is typical of the deep Eastern Pacific (WOCE,
120 www.nodc.noaa.gov/WOCE; Sector p17N, St. 10, 38.23733N, 124.93833W, 3087 m depth;
121 DIC=2352.7; TA=2442.3; pH_{tot}=7.7834; $Omega_{CA}$=0.93; $Omega_{AR}$=0.6).
122 As part of each experiment's termination, suites of samples were obtained from a site
123 with no increase in CO_2 (i.e., ~100 m from CO_2 release), from inside the circle of PVC cylinders,
124 and from inside individual PVC cylinders. These three treatments are hereby referred to as
125 Control, Elevated, and Direct Contact, respectively. Elevated treatment samples were further
126 distinguished depending on their distance from the PVC cylinders due to experimental logistics
127 (see below): Elevated Center samples were collected from the approximate middle of the PVC-
128 cylinder circle while Elevated Edge samples were obtained close to PVC cylinders (i.e., within
129 ~1-3 m; Fig. 1). The configuration of core locations was mandated by the fact that this
130 experiment included sampling for multiple additional purposes and by the logistical
131 considerations of working in the deep sea. For example, the ROV manipulator reach and
132 seafloor disturbance in the form of sediment resuspension were critical factors for core
133 placement. More details on the experimental design and concurrent science objectives are
134 presented in Ricketts *et al*. (2005; *submitted*) and Thistle *et al*. (2007).
135 The fluorogenic probe CellTracker™ Green CMFDA (5-chloromethylfluorescein
136 diacetate; Invitrogen, hereafter referred to as CellTracker Green) was used to distinguish living
137 from dead foraminifera (Bernhard *et al*., 2006) and, thus, establish survivorship. A fluorogenic
138 probe is a non-fluorescent compound that yields a fluorescent product after structural
139 modification; CellTracker Green accumulates intracellularly in live cells after enzymatic

140 hydrolysis cleaves the molecule, thereby producing fluorescence (reviewed in Bernhard *et al*.,
141 1995).

142 Quantitative sediment samples were obtained using pushcores outfitted with a device to
143 allow injection of concentrated CellTracker Green after pushcore placement into the seafloor.
144 This approach was adopted to allow in situ seafloor incubations with CellTracker Green because
145 temperature and pressure changes during ascent could cause mortality due to sample recovery
146 rather than due to in situ experimental conditions. CellTracker Green is soluble in dimethyl
147 sulfoxide (DMSO); this stock solution was diluted with an equal volume of 0.2 μm-filtered
148 seawater (FSW) to prevent freezing in the injector capillary tubing prior to dispensation.

149 Initially, the pushcores (7-cm inner diameter) were emplaced into sediments (Fig. 2a) so
150 that the internal header space volume was within a targeted range. To do this, the pushcorer was
151 positioned so that it penetrated the seafloor to a depth placing the sediment-water interface
152 between two core-tube markings. The ROV manipulator arm was then used to carefully squeeze
153 a large diameter (2-cm outer diameter) flexible tube filled with FSW, which flowed through a
154 check valve to displace the concentrated CellTracker Green from the capillary tubing producing
155 a final concentration of ~1 μM CellTracker Green within each core's header space. These cores
156 incubated in situ until the following day, when they were recovered by the ROV. A negative
157 control core, which was injected with DMSO and FSW but not CellTracker Green, was similarly
158 incubated and collected from an area with no increase in CO_2, during Experiment 2. To maintain
159 the integrity of the sediment-water interface, the pushcores were not equipped with a stirring
160 device.

161 After ROV recovery by the support ship, the incubated pushcores were taken within ~10
162 minutes into an environmental room approximating in situ temperature (~5 ^{o}C). Within an hour,

163 the overlying water containing CellTracker Green was removed via siphon; the surface 1-cm of
164 each core was sectioned from the underlying sediments, placed in a high density polyethylene
165 (HDPE) container, and fixed in ~3.8% formalin buffered with sodium borate. All Direct Contact
166 treatment cores became disturbed upon ascent due to degassing. In these instances, fine particles
167 were suspended and the sediment-water interface was typically disturbed. Thus, for these cores,
168 the overlying waters were retained in addition to the surface sediment interval or allowed to
169 settle for approximately an hour prior to sectioning.

170 In the shore-based laboratory, samples were sieved over a 63-μm screen with tap water,
171 and the coarser fraction examined with a Leica MZ FLIII stereo dissecting microscope equipped
172 with appropriate epifluorescence optics (excitation 480 ±20 nm, emission ≥510 nm). Fluorescent
173 foraminifera were isolated, segregated into brightly and dimly fluorescent groups, sorted by
174 species within those groups, and enumerated. Thecate and "soft-shelled" agglutinated taxa,
175 which were not identified to species, were archived in buffered formalin while calcareous and
176 robust agglutinated taxa were air dried and archived on micropaleontology slides. Because a
177 single individual of observed unilocular cylindrical agglutinated foraminiferal taxa (i.e.,
178 *Bathysiphon*, *Rhabdammina*, *Rhizammina*) can easily break, those fragments were minimally
179 enumerated to prevent over estimates of population density. Within a sample, it is typically clear
180 which fragments likely arose from the same specimen due to test grain size, diameter,
181 composition, texture, and color. During the picking process, the treatment of each sample (i.e.,
182 Control, Elevated, Direct Contact, negative control) was withheld from the microscopist.
183 Species counts were normalized to in situ sample volume to provide abundance estimates.

184 Abundance data from each experiment were statistically analyzed separately for each
185 taxonomic group. For thecate and agglutinated groups in Experiment 1, a one-way ANOVA was

used to test for differences in density among the Control, Elevated Center, Elevated Edge, and Direct Contact treatments. For Experiment 2, a one-way ANOVA was conducted to test whether mean density of either group differed between the Control, Elevated Edge, and Direct Contact treatments. To homogenize variances, analysis was done on logged transformed thecate data and on square root agglutinated data.

To determine the effects of CO_2 injection on calcareous foraminiferal abundance, the mean abundance in the Control treatment was compared to the mean abundance in the Direct Contract treatment. For this comparison, the mean of the Control treatment was compared to a value of 0 under the null hypothesis with a one sample t-test because calcareous foraminifera were absent in the Direct contact treatment cores. Additionally, a one-way ANOVA was used to test whether mean densities of calcareous foraminifera differed between Control, Elevated Center and Elevated Edge treatments. For Experiment 2, mean calcareous foraminiferal densities in the Elevated Edge and Controls were compared with a two sample t-test and the mean density of the Control treatment was tested in a single sample t-test with a hypothesized mean of zero.

Results

Only cores that were visibly injected (Fig. 2a) were processed and analyzed. In one case, the capillary tubing parted from a coupling, thus CellTracker Green was not injected into that core so it was excluded from study. Replicate cores from Control and Elevated treatments of each experiment were typically taken within 1 m of each other (Fig. 2b); Direct Contact cores were obtained from different PVC cylinders. Careful manipulation by the ROV pilots resulted in absence of visible sediment-water interface disturbance within the corers, except for those collected from within the PVC cylinders (i.e., Direct Contact cores), which degassed during ascent as noted above.

The negative control core (i.e., injected solely with DMSO, without CellTracker Green) lacked brightly fluorescent foraminifera, but had dimly fluorescent foraminifera. Thus, only brightly fluorescent foraminifera were considered to be living at the time of seafloor incubation with CellTracker Green. In CellTracker Green-incubated cores, the CellTracker Green diffused to at least 1-cm depth because some organisms in the 1-2 cm interval fluoresced brightly.

pH changes

The perturbations of pH measured in bottom waters during both experiments were variable but not large. In an area equivalent to the Elevated Edge treatment, the maximum pH reductions compared to ambient [pH reductions were calculated as perturbations from the background pH measured at the site] values were 0.15 to 0.25 pH units, with average changes less than 0.05 units. In the center of the circle (i.e., Elevated Center treatment), pH reductions sometimes approached 0.1 – 0.2 pH units, but typical pH perturbations were less than 0.05 units.

In areas corresponding to the Elevated Edge treatment, pore-waters of surface sediments at the beginning of Experiment 2 (i.e., 1-3 d after hydrate placement) were ~0.2 units lower than

224 at control locations (~50 m away) but the pH of Elevated Edge pore-waters at experimental
225 termination did not differ from the pore-water pH of control area sediments (Barry *et al*.,
226 *submitted*). Changes in the pH of surficial sediment pore waters (i.e., upper ~10 cm) were large
227 within Direct Contact cylinders (~ -2.0 pH units compared to pore-water of control sites; Barry *et*
228 *al*., *submitted*).

229 *Species composition of living foraminiferal assemblages*

230 The living calcareous foraminiferal assemblage, as determined by bright CellTracker
231 Green labeling, in the Control and Elevated samples was dominated by rotaliid forms including
232 *Uvigerina canariensis*, *Chilostomella oolina*, and *Globobulimina pacifica*; the most common
233 miliolid form was *Quinqueloculina venusta* (Table 1; Fig. 3). The living non-calcareous
234 foraminiferal assemblage in Control and Elevated samples was dominated by thecate and "soft-
235 shelled" agglutinated forms such as saccamminids, as well as *Reophax dentaliniformis*, *R.*
236 *spiculifer*, *Hormosinella guttifera*, and *Paratrochammina challengeri* (Fig. 4). Agglutinated
237 species such as *R. dentaliniformis* and *R. spiculifer* and thecate species dominated Direct Contact
238 samples.

239 *Abundances of live foraminifera*

240 The foraminiferal abundance between cores within a treatment was highly variable for
241 some treatments but not for others (Table 1). Abundances of all live foraminifera ranged from
242 4.4 to 5.7 specimens·10cm^3 in Initial Control cores of Experiment 1, and from 6.2 to 11.2
243 specimens·10cm^3 in Control samples collected at the end of either experiment (Supplemental
244 Data; Fig. 5). Thus, the average abundance of foraminifera in Control samples was 7.7
245 specimens·10cm^3 (n = 8, SD = 3.0). Foraminiferal abundances within the Elevated samples
246 spanned a wider range (2.6 to 26.0 specimens·10cm^3; $\bar{x}$ = 12.1, SD = 8.6) than those within

247 Direct Contact samples (1.3 to 11.7 specimens·10cm^3; $\bar{x}$ = 5.4, SD = 4.0). The average of total
248 abundance of Elevated Edge samples ($\bar{x}$ = 14.9, SD = 10.4) was higher than all other treatments
249 (Fig. 5a,b).
250 Abundances of thecate foraminifera averaged 2.3 specimens·10cm^3 (SD = 2.3) in all
251 Control cores and 3.3 specimens·10cm^3 both in Elevated samples and Direct Contact samples
252 (SD = 3.8, 2.5, respectively). Abundances of agglutinated foraminifera averaged 2.1-2.2
253 specimens·10cm^3 in both the Control and Direct Contact samples, but were nearly twice as
254 abundant in samples collected from within the circle (i.e., Elevated treatments combined; 4.1
255 specimens·10cm^3, SD = 3.8). The mean abundances of thecate and agglutinated groups were
256 generally higher in Elevated Edge samples than in other treatments (Fig. 5c-f), the exception
257 being thecate abundances for Experiment 2 (Fig. 5d). The mean density of thecate or
258 agglutinated foraminifera did not differ significantly between the Control, Elevated Center,
259 Elevated Edge or Direct Contact treatments in Experiment 1 (One-way ANOVA, thecate, $F_{3,7}$ =
260 0.39, p = 0.762; agglutinates $F_{3,7}$ = 0.49 p= 0.701) or in Experiment 2 between Control, Elevated
261 Edge, and Direct Contact (One-way ANOVA, thecate, $F_{2,3}$ = 0.09, p = 0.913; agglutinates, $F_{2,3}$ =
262 0.40, p = 0.699).
263 Calcareous foraminiferal abundances averaged 3.2 specimens·10cm^3 and 4.7
264 specimens·10cm^3 in Control and Elevated cores, respectively, but no calcareous foraminifera
265 were living in the Direct Contact cores (Table 1; Fig. 5g,h). For Experiment 1, the mean density
266 of calcareous foraminifera did not differ significantly between the Control, Elevated Center or
267 Elevated Edge treatments (One-way ANOVA, $F_{2,5}$ = 0.48, p=0.645) and, for Experiment 2, their
268 mean density did not differ significantly between the Control and Elevated Edge treatments
269 (Two sample t-test, -1/x transformed data, df = 2, t = 2.88, p = 0.102). In both experiments, the

mean density of calcareous foraminifera in the Control samples was significantly different from a mean of zero (One sample t-test, Experiment 1: $t = 6.26$, $df = 2$, $p = 0.025$, Experiment 2: $t = 25.96$, $df = 1$ $p = 0.025$). Thus, there were significantly more calcareous foraminifera in the Control treatments than in the Direct Contract treatment, which lacked calcareous foraminifera in every core of both experiments.

Discussion

The hypothesis that higher mortality would occur in calcareous foraminifera compared to thecate and agglutinated foraminifera in response to deep-sea CO_2 sequestration is supported by our data. Survivorship of the thecate and agglutinated foraminiferal populations at our Monterey Bay sites were not significantly affected by direct exposure to CO_2 hydrate on the experimental time scale.

Although the water depth at our experimental sites likely exceeded the regional calcite saturation depth and the carbonate compensation depth (CCD), calcareous (calcitic) foraminifera were living in the area as evidenced by Control treatment results. The existence of calcitic foraminifera is not unexpected since most abyssal regions of today's oceans have live calcareous foraminifera (e.g., Bernhard, 1992; Linke & Lutze, 1993). In general, the observed abundances of foraminifera in control samples were comparable to abundances from similar water depths at sites ~150 km to the south (Bernhard, 1992). Using a different viability method (i.e., Adenosine Triphosphate (ATP) assay), abundances of live foraminifera integrated over the surface 1 cm ranged from 0 to ~12 specimens·10cm^3 in water depths from 3319-3728 m ($\bar{x}$ ~6 specimens·10cm^3; n = 4; Bernhard, 1992). Deep-sea benthic foraminiferal distributions are known to be patchy on the scale of km to cm (e.g., Bernstein *et al*., 1978; Bernstein & Meador, 1979), so it is not unusual to document considerable variations in foraminiferal abundance over short distances. Indeed, Bernhard (1992) also noted large variations in foraminiferal abundances between sites, although those cores were separated by kilometers, not meters, as in this study. The species compositions of agglutinated and calcareous assemblages encountered in our samples resemble those previously reported from the region at comparable water depths (e.g., Bernhard, 1992).

298 Even though some bathyal and abyssal foraminiferal species' abundances exhibit
299 subsurface maxima (e.g., Corliss, 1985; Bernhard, 1992), a down-core analysis of our samples
300 was not feasible. Because, in general, the majority of bathyal foraminifera live in the top cm
301 (e.g., ~52-71%, Gooday, 1986; ~80%, Szarek *et al*., 2007) and because we expect the maximum
302 physical and chemical changes due to CO_2 hydrate release within the surface sediments, we feel
303 our data reflects the typical survival response of bathyal benthic foraminifera to such
304 environmental perturbations. It is possible that foraminiferans migrated in response to the
305 changing milieu, as observed in other experimental studies (e.g., Alve & Bernhard, 1995;
306 Moodley *et al*., 1998). In particular, if infaunal specimens migrated upward into the surface cm,
307 the average abundances in treatments affected by CO_2 would have been higher than those of
308 Controls. Indeed, the abundance data for agglutinated taxa were consistently higher, although
309 not significantly different, in Elevated Edge samples compared to Controls (Fig. 5e,f). This
310 trend was not evident for other groups (Fig. 5c,d,g,h). Why foraminifera may have migrated
311 toward potentially stressful concentrations of CO_2 is unclear, except the option of migrating
312 deeper into sediments would have been more stressful for aerobic migrants compared to
313 facultative anaerobes, which are known for foraminifera (e.g., Bernhard & Alve, 1996; Moodley
314 *et al*., 1998), or if the migrants were incapable of performing complete denitrification (Risgaard-
315 Petersen *et al*., 2006; Høgslund *et al*., 2008). Recent findings suggest elevated pCO_2 exposure is
316 stressful to at least some meiofauna because higher numbers of harpacticoid copepods emerged
317 from sediments in response to elevated pCO_2 compared to copepod emergence rates at control
318 sites (Thistle *et al*., 2007).
319 Degassing of cores collected from PVC cylinders during ascent may have minimized the
320 observed foraminiferal abundances in the Direct Contact cores. We discount this possibility

because the average density of thecate and agglutinated morphotypes in Direct Contact samples was near or equal to their density in Control cores. If significant abundance dilution occurred due to degassing disturbance, we would expect all three groups of foraminifera to be similarly affected, which was not the case.

Observed patterns and magnitudes of pH change resemble those reported for similar experiments (Barry *et al*., 2005). Importantly, the observed bottom-water pH decreases in areas corresponding to Elevated Center treatments (~ 0.05 units for Experiment 1) were small compared to those declines corresponding to Elevated Edge treatments (initially 0.2 pH units, but ~ 0.1 to 0.15 pH units at experiment end). Thus, it is not surprising that all three groups of benthic foraminifera (i.e., calcareous, agglutinated, thecate) tolerated the Elevated Center treatments because those sediments apparently did not experience extended large decreases in pH and, by inference, extended large increases in pCO_2. It is also noteworthy, however, that thecate and agglutinated foraminiferal survival was not negatively impacted by the substantial pH decrease (> 1 pH unit) in the Direct Contact treatments.

Economically viable protocols of deep-sea CO_2 sequestration include formation of deep-sea CO_2 lakes and near-bottom injection of CO_2 (reviewed in Herzog *et al*., 2000; Caldeira & Akai *et al*., 2005). In all cases, a plume of CO_2-rich seawater will lead from liquid CO_2 or CO_2 hydrate drifting with currents and diffuse, eventually being diluted (Caldeira and Akai *et al*., 2005). Thus, CO_2 and pH gradients will range from substantial (e.g., the lowest pH measured is 4.5, a value measured within cm of CO_2 hydrate; Brewer *et al*., 2000) to mild (i.e., <-0.05 pH units) at some distance, depending on specific mixing conditions. Our Direct Contact treatment recreates the CO_2 lake approach, although on a much smaller scale. Benthos beneath any CO_2-hydrate pool will be directly exposed for long periods of time unless they can migrate from the

hydrate pool (Tamburri *et al*., 2000). Deep-sea foraminifera move very slowly (<25 $\mu m \cdot min^{-1}$; Gross, 2000), so active avoidance at these spatial scales is an implausible escape mechanism for them. The pH changes in our experiments are similar to those under any carbon sequestration option: conditions of our Elevated treatments are within the magnitude of pH change expected along the dilution gradient resultant from presently proposed mitigation scenarios.

The in situ response of foraminifera (and any other group of deep-sea organisms) to such CO_2-hydrate exposure is unknown over time periods longer than our experiments. Our results indicate that although the calcareous foraminiferal population tolerates short exposures to a 0.2 pH unit decrease, their survival response to direct CO_2-hydrate exposure results in a significant increase in mortality. If, however, plumes of dissolved CO_2 hydrate are diffused from the seafloor over a shorter time scale than our experiments, it is possible that calcareous foraminiferal survival will not be significantly impacted by large-scale CO_2 sequestration.

Although the effects of localized deep-sea CO_2 sequestration appear to negatively impact calcareous foraminifera (Ricketts *et al*., 2005; *submitted*; this study), two other protistan groups (i.e., flagellates and amoebae; Barry *et al*., 2004), and a number of metazoan meiofauna (Barry *et al*., 2004; Carman *et al*., 2004; Watanabe *et al*., 2006; Fleeger *et al*., 2006; Thistle *et al*., 2007), our findings suggest that survival of at least some protistan meiofauna (i.e., thecate and agglutinated foraminifera) are not similarly influenced by direct exposure to CO_2 hydrate. Because the abundance and diversity of thecate and agglutinated foraminifers are considerable in bathyal and abyssal sediments (e.g., Gooday *et al*., 1998; 2000; Smith *et al*., 2002) and their abundance can exceed that of other meiofauna (e.g., Gooday et al 2000), at least one major group of deep-sea meiofauna will likely not collapse if large-scale sequestration of CO_2 is implemented on the deep-ocean floor. Furthermore, our data showing that thecate and agglutinated

367 foraminiferal abundances do not significantly decline in response to direct exposure to CO_2
368 hydrate at these spatial and temporal scales substantiate recent laboratory findings documenting a
369 shallow-water thecate foraminiferan species survives ~2-week exposure to extremely high pCO_2
370 (200,000 ppm) where some specimens even reproduced (Bernhard et al., *in press*).

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Table 1. Mean abundances of live foraminifera [(#·10cm^3); listed for all foraminifera and by foraminiferal group] for each treatment, presented by experiment. The number of replicates (# cores) is also listed. SD = Standard deviation.

Treatment	Experiment	# cores	Mean (SD)			
			Total	Thecate	Agglutinated	Calcareous
Initial Control	1	3	4.8 (0.8)	0.9 (0.5)	1.6 (0.8)	2.3 (0)
Control	1	3	9.5 (2.9)	3.3 (2.8)	3.0 (0.8)	3.2 (1.8)
	2	2	9.4 (1.8)	2.9 (3.3)	2.0 (0.9)	4.6 (0.5)
Elevated Center	1	3	8.3 (4.5)	2.4 (2.2)	3.0 (2.6)	2.9 (1.6)
Elevated Edge	1	2	14.3 (16.6)	6.5 (7.0)	6.1 (7.5)	1.7 (2.0)
	2	2	15.6 (6.9)	1.4 (0.6)	3.6 (1.5)	10.5 (5.0)
Direct Contact	1	3	4.7 (1.7)	3.0 (0.9)	1.7 (1.4)	0
	2	2	6.5 (7.4)	4.0 (4.6)	2.5 (2.8)	0

Figure Legends.

Figure 1. Schematic representation of experimental configurations (a. Experiment 1; b. Experiment 2). The CO_2 cylinders are shown as gray circles, and CTDs equipped with pH sensors are shown as squares. Pushcores analyzed for this study are shown as smaller circles; pushcore treatment categorizations are: open = Control; / = Initial Control, black = Direct Contact, + = Elevated Center, * = Elevated Edge; ~ = DMSO.

Figure 2. Photographs of injector pushcores placed in the seafloor. a. Representative pushcore from Elevated Center treatment immediately after the ROV manipulator released its grip on the thick injector tubing (upper left). Inset: Note the stream of whitish fluid (i.e., CellTracker Green and DMSO) entering the header space from the capillary tubing, which extends through the corer top, approximately to the arrow. b. Suite of three Control pushcores incubating in situ during Experiment 1.

Figure 3. Reflected light (a, c, e, g) and corresponding epifluorescence (b, d, f, h) micrographs of calcareous foraminifera collected at experimental termination. Those labeled with * were from a Direct Contact treatment core (935-JB2); those without * were from a Control treatment core (937-JB7). Note that none of the Direct Contact specimens fluoresce brightly. a, b. Top row: *Quinqueloculina venusta* , bottom row: *Pyrgo murrhina*; c, d. *Cassidulina* cf. *delicata*; e, f (left to right): *Hoeglundina elegans, Cibicidoides* sp.; g, h. *Uvigerina canariensis*. Scale bars: 100 μm.

Figure 4. Reflected light (a, c, e, g) and corresponding epifluorescence (b, d, f, h) micrographs of agglutinated and thecate foraminifera collected at experimental termination. Those labeled with * were from Direct Contact treatment cores (935-JB2 or 935-JB3); those without * were from Control treatment cores (937-JB6 or 937-JB7). Note that the Direct Contact specimen in c,d fluoresces brightly; other Direct Contract specimens were deemed dead. a, b (left to right). Unidentified allogromiid, saccamminid; c, d (left to right). *Pelosina* sp., unidentified allogromiid; e, f. *Hormosinella guttifera*; g, h. *Veleroninoides wiesneri*. Scale bars: 100 μm.

Figure 5. Histograms of CellTracker Green labeled benthic foraminifera, presented as total density (specimens·10cm^3) and by group and treatment for each Experiment. Error bars reflect standard error.

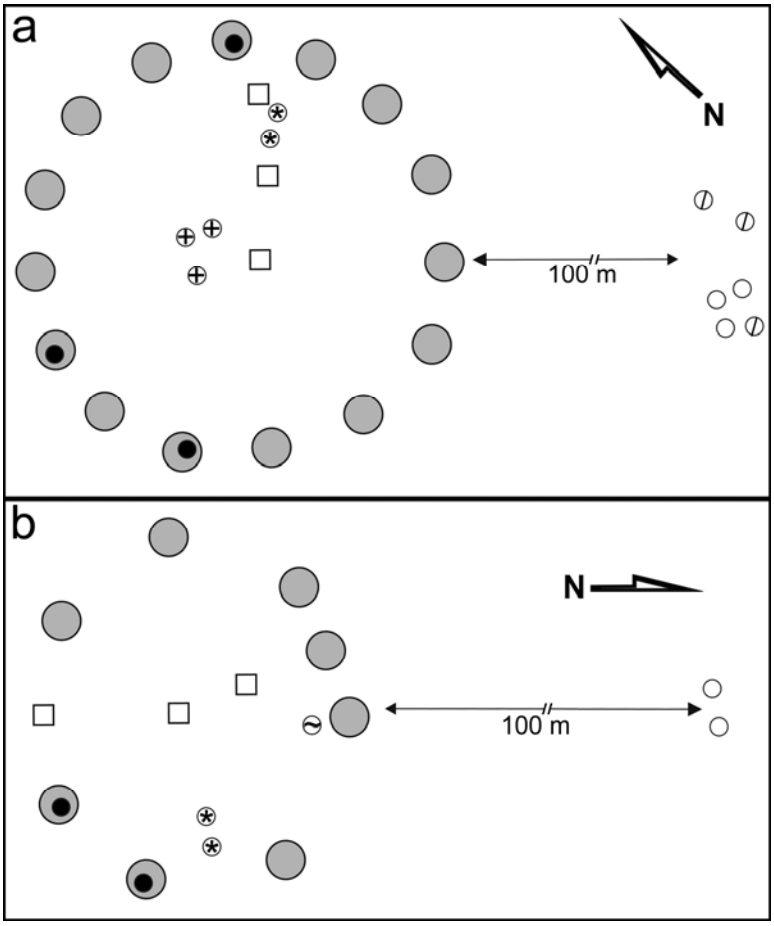
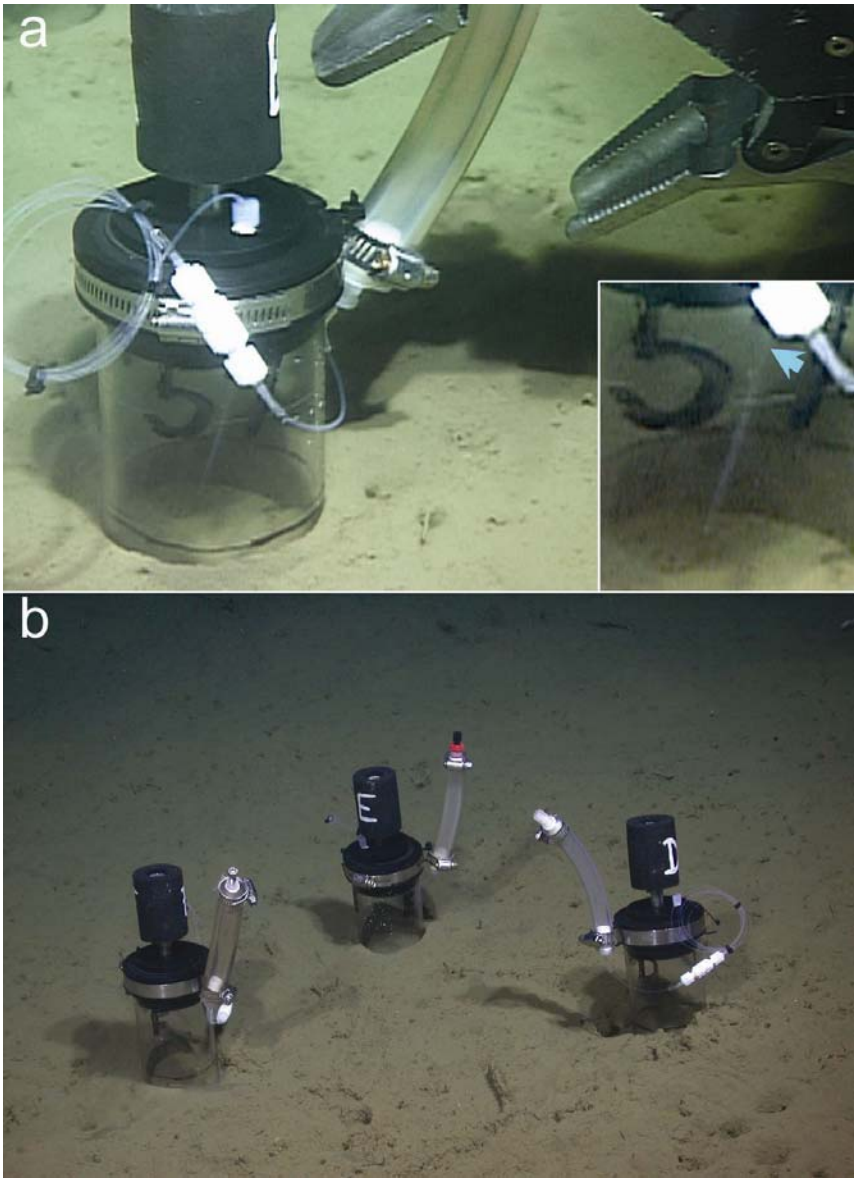


Figure 1.



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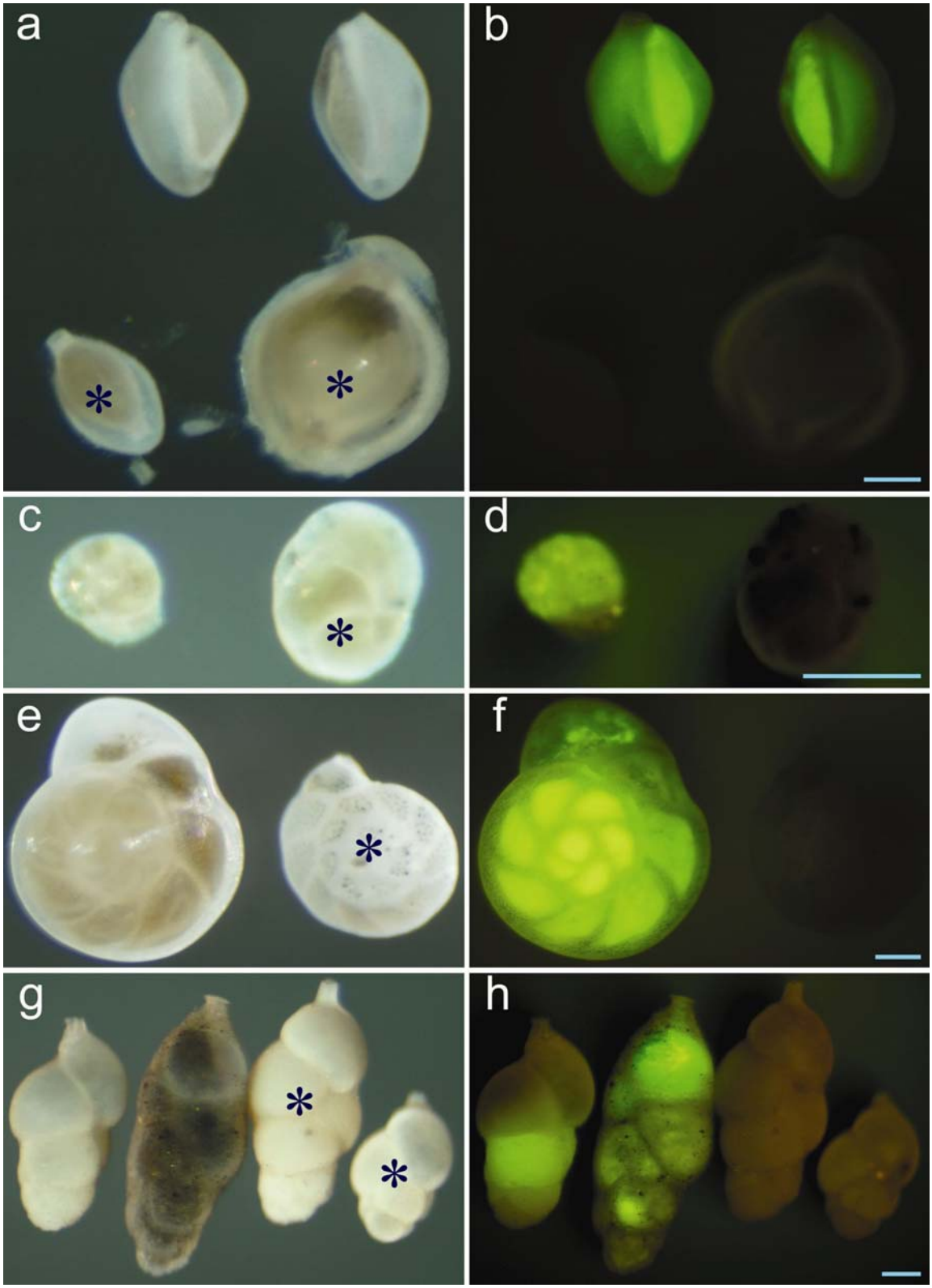
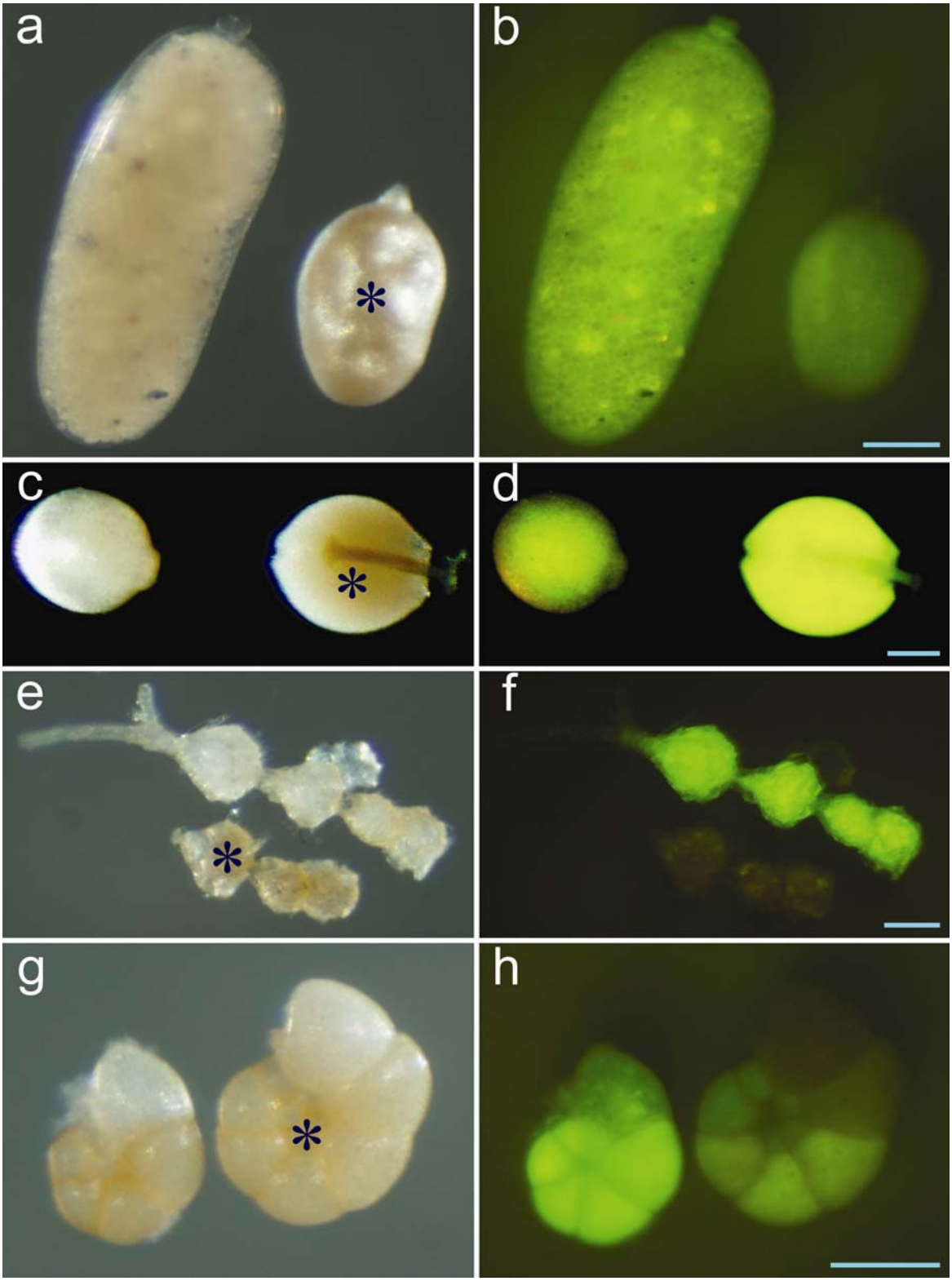


Figure 3.



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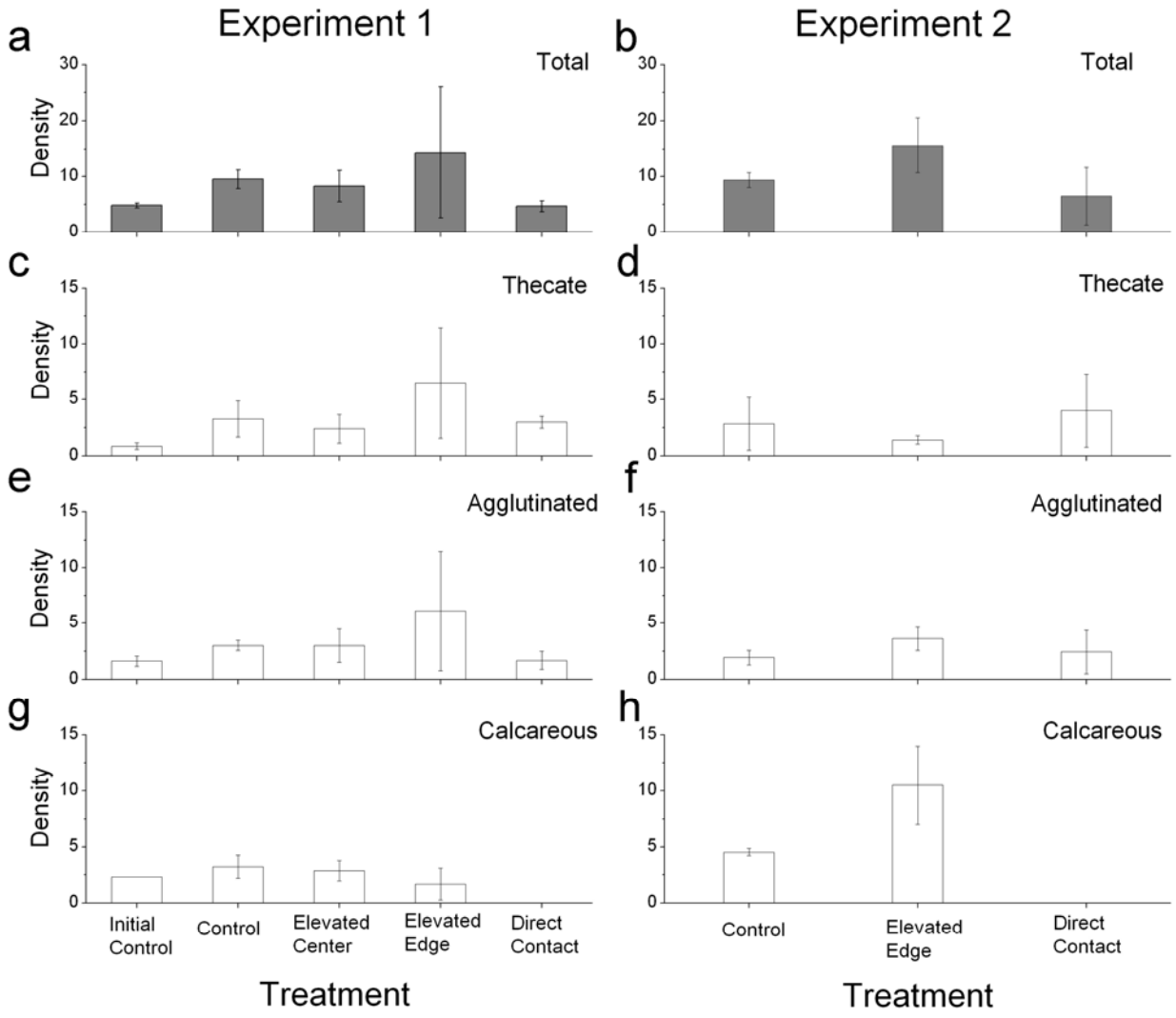
565 Figure 4.



566

567 Figure 5.